Embryonic Development and Post-Hatching Survival of the Sepiolid Squid *Euprymna scolopes* under Laboratory Conditions

BY

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(6 Plates)

INTRODUCTION

RECENTLY THERE HAS BEEN renewed interest in the development and particularly the rearing of cephalopods. CHOE (1966) reared three species of *Sepia, Sepioteuthis lessoniana* and *Euprymna berryi* to adult size from the egg. RICHARD (1966) and SCHRÖDER (1966) successfully reared *Sepia officinalis* and LA ROE (personal communication and reported in "Sea Secrets," 1970) reared *Sepioteuthis sepioidea*. BOLETZKY *et al.* (1971) recently reported successful laboratory rearing of five species of *Sepiolinae*. Among the Octopoda, ITAMI *et al.* (1963) were able to rear *Octopus vulgaris* through the planktonic stage and BOLETZKY & BOLETZKY (1969) were able to successfully raise a single specimen of *Octopus joubini* from hatching to egg laying. The obvious goal of these rearing experiments is to establish a laboratory reared cephalopod for experimental purposes and to form a background for the development of a potential human food source.

This report briefly describes the embryonic development of *Euprymna scolopes* (Berry, 1913), a Hawaiian sepiolid, and early post-embryonic development. The development of this species is remarkably similar to that of the loliginid *Loligo pealei* (Lesueur, 1821), and it is possible to use the same staging criteria (ARNOLD, 1965a). Since there presently is no published photographic record of the development of this species, such a paper is amply justified to form the basis for the publication of experimental results.

MATERIALS AND METHODS

The adult animals used in this study were collected on sand and mud flats on the southeastern shore of the island of Oahu during evenings of low tide. By using a lantern it was possible to capture swimming squid with dip nets as they foraged for food in water less than a half meter in depth. Usually the animals paid no attention to the light and showed avoidance behavior only when they were disturbed by the dip nets. Once captured they were transferred to large polyethylene bags of sea water, transported to the laboratory, and kept individually in polyethylene tanks 45 cm × 31 cm, filled with sea water to a depth of 25 cm. Each tank had about 3 cm of sand on the bottom and was equipped with subgravel filters. The water in each tank was changed twice weekly. In some cases the tanks were divided in half with a slotted clear plastic sheet to facilitate holding more animals. The squid were routinely fed one adult shrimp, *Leander debilis,* each night, but occasionally small *Gambusia affinis* were also offered and accepted, but the squid exhibited a strong preference for the shrimp. On a few occasions where more than one individual of dissimilar size were placed in the same tank cannibalism was evident.

The eggs were kept under much the same conditions, but the egg masses were separated into two portions, one of which was left *in situ* while the other was suspended on a string to facilitate observation and manipulation. Observations were made randomly selecting a few eggs daily and mechanically removing the white outer coat of the egg capsule and as much jelly as possible to facilitate visibility. Once observed and photographed with a dissecting microscope the eggs or embryos were fixed in Bouin's solution to avoid possible introduction of abnormalities caused by manipulation or overheating. Those eggs left *in situ* were disturbed as little as possible and served as a control for time of hatching and degree of spontaneously occurring abnormalities. The stages of development used in this paper are those of ARNOLD (1965a).

The hatched animals were also kept in the polyethylene tanks, but in this case a layer of fine screening was placed

over the subgravel filter and fine sand was put on the tank bottom. Several individuals were kept together in the same container. The young squid were fed at liberty on larval and adult mysids, *Anisomysis* sp., adult *Artemia salina,* and occasionally newly hatched *Octopus cyanea* larvae. To increase their chances of survival, the young animals were disturbed as little as possible, but on a few occasions randomly chosen representative animals were selected for microscopic examination and photography. These animals were returned to the tank and seemed unaffected by the brief disturbance.

OBSERVATIONS

The eggs and embryos described here are from two batches of eggs laid on March 7, 1969 and March 11, 1971 as well as from eggs collected in the field in the spring of 1970. In the eggs laid in the laboratory it was possible to closely follow development from first cleavage onward to hatching and beyond. The females that laid the eggs in the laboratory were collected on February 10, 1969 and February 22, 1971 and were isolated from all other squid until the time of laying. Apparently, successful copulation had occurred sometime before collection and the sperm stored until laying. In the 1969-eggs, fertility was low (11%), but in the 1971-eggs almost 100% were fertile and began development more or less synchronously. Since egg laying occurred at night with no prior indication, it was not possible to observe the actual process of egg deposition, but it was possible to estimate the time of fertilization from observations on eggs artificially inseminated by techniques previously described for *Loligo* (Arnold, 1971a). In these eggs, the first cleavage furrow appeared about 8 hours after fertilization; therefore, the naturally laid eggs were probably fertilized in the middle of the night. Since fertilization and egg laying are synchronous in all of the known cephalopods, it is probable egg laying also occurred in the middle of the night. In the 1969 laying, the female laid 152 eggs the night of the 6th and 7th of March, but also laid 4 smaller infertile batches later. Following this she died on April 17. The female who deposited the 1971-egg mass died without further laying 26 days after laying. In the 1969-female, the gonad was completely spent, but the 1971-female laid 164 eggs at once but still had approximately 130 eggs in her gonad at the time of death.

Figure *1* shows the appearance of part of the 1971 egg mass. The individual eggs were surrounded with several layers of a semitransparent jelly and covered with a thin, opaque, white flexible capsule. The eggs in the mass were cemented to each other by the white capsule material and to the side of the tank several centimeters above the sandy bottom. Interspersed among the outermost eggs were grains of sand and small bits of coral, apparently cemented in place with the same leathery opaque white material. In nature the egg masses occur on rocks or loose pieces of coral in the same areas occupied by the adults. As the embryos developed, the pigmentation of the eyes became evident through the gradually clearing capsule wall. The eggs themselves were contained singly inside a tight fitting chorion and at the time of first cleavage measured 2 by 1.6 mm and had an ovoid shape (Figure *2*). When the egg jelly was removed or eggs taken directly from the gonad, the large micropyle was evident at the narrow end of the egg (animal pole). In some eggs the chorion was quite sculptured with whirls and ridges, but in other eggs of the same batch the chorions were smooth and undecorated (Figures *3*E and *3*F). During development the chorion expanded somewhat but not as much as is common in *Loligo* (Arnold, 1965a).

Figures *2*, *3*, *4*, and *5* show the development of *Euprymna* until the time of hatching. Figures *2*A and *2*B show formation of the first cleavage furrow. Prior to this, there was considerable cytoplasmic streaming to form a large blastodisc of granular, yellowish cytoplasm beneath the micropyle. The polar bodies were evident only with careful examination and were not successfully photographed. As in all the known cephalopods, the egg is highly telolecithal and the early cleavage pattern is quite regular (Figures *2*C, *2*D, *2*E, *2*F, *2*G) (*cf. Loligo,* Arnold, 1971a), but because of an apparently stronger contraction of the furrow bases in combination with the undercutting furrow some unusually shaped cells are sometimes seen (Figure *2*E). Cleavage proceeded until a small blastoderm was established with lines of cells derived from the "blastocones" (Vialleton, 1888) radiating from its edge (Figure *2*G). Eventually these lines of cells are obliterated by the increased diameter of the growing blastoderm.

The segregation of the germinal layers (stages 10 and 11; Figures *2*H and *2*I) appears to be similar to other cephalopods (Arnold, 1971b) except folds develop in the cellular layers near the margin of the blastoderm. Occasionally an abnormality in which marginal cells penetrate downward into the yolk rather than going under the outer layer occurs at this time. Similar abnormalities have been observed in many other species at this stage of development. None of these embryos develop normally although they continue to survive for a few days. Normally, after the cellular layers are established, the cells continue to expand and encompass the yolk mass (Figures *2*J and *2*K) so by the time of organogenesis the resultant embryo is essentially composed of three layers: an innermost syncytial periblast (=yolk epithelium of the early literature);

an outer layer of cells; and an intermediate layer relatively distinct from the two other layers.

Figure *3* shows the sequence of organogenesis which is comparable to other cephalopods and will not be dealt with in detail here. A detailed description of cephalopod ontogeny has recently been published elsewhere (Arnold, 1971b). For convenience the figure captions should serve as an anatomical guide. One striking exceptional feature is the shape of the external yolk sac and its relation to the embryo proper. As the embryo increases in size, the yolk sac becomes bilobed and bends vertically to underlie and partially surround the developing head. This may be due, in part, to the rather closely applied chorion in early development, but this shape persists until hatching when the external yolk sac is completely consumed.

The internal yolk sac is also somewhat exceptional in that it is multilobed rather than bilobed as in *Loligo* or *Octopus* and becomes quite large in later stages of development (Figures *3*J, *4* and *5*). After hatching the internal yolk sac diminishes until it is finally completely digested (Figures *6*E and *6*F) at which time the squid is already feeding. The "T"-shaped organ of Hoyle appears much earlier (stage 26) than in either *Loligo pealei* or several species of Octopus. It becomes quite prominent until hatching and rapidly disappears after hatching is completed (Figures *6*E and *6*F). Some pigmentation occurs earlier than in *L. pealei,* the eyes being noticeably orange at stage 23; and yellowish iridophores appear around the eye at stage 28 (Figure *4*I). The fins are prominent and muscular, and the embryo is able to flap them from about stage 26 onward, although most of the movement within the chorion is caused by mantle contraction.

Hatching could be stimulated by removal of the capsule and outer layer of jelly at stage 29 (18 days development at 24° C) but spontaneous hatching did not occur until the external yolk sac was considerably reduced (20 days). Hatching seemed to involve a number of sequential steps when the embryos were stimulated. When the outer shell of the capsule was removed, the embryo contracted violently and remained contracted for a period of several seconds to a few minutes. This was followed by a period of rapid movement within the chorion caused by rapid pumping of the mantle and movement of the arms. At times the suckers appeared to be definitely attached to the inside of the chorion. The animal then stretched the mantle and pushed with the arms so the organ of Hoyle was actively forced against the chorion. The organ of Hoyle seemed to attach to the inside of the chorion. In a few seconds, the chorion outside the organ of Hoyle was digested and the jelly layer was quickly dissolved. The larva escaped by pushing with its arms on the far side of the chorion and by active swimming motions of the mantle. Presumably intrachorionic pressure helped to force the larva out because after hatching the chorion was reduced in volume and its walls were no longer smooth (Figure *6*C).

A total of 26 individuals successfully hatched and were placed in a tank in an attempt to rear them to maturity. They were provided with living larval and adult *Anisomysis* sp. and after the first day, some began to actively feed and attempt to conceal themselves in the sand. Similar behavior has been described in *Sepia* by Wells (1962). Apparently, some individuals never fed because only 10 squid survived. Their growth was extremely rapid as is illustrated in Figures *6*F, *6*G and *6*H. Because we were reticent to disturb the animals any more than absolutely necessary, we did not attempt to measure them and construct a growth curve. However, in one randomly selected representative used for photography, the mantle length increased from 1.6 mm at hatching to 2.4 mm after 11 days, to 3.3 mm after 22 days and to 4.8 mm after 28 days. This rate of growth is comparable to the growth curves for *Sepiola* and *Sepietta* reported by Boletzki *et al.* (1971). At the time of this writing the 10 animals are all still alive and growing rapidly.

DISCUSSION

It would appear that *Euprymna scolopes* will prove to be a valuable cephalopod for developmental, physiological, behavioral, and possibly genetic studies. The ease with which the animals can be maintained under laboratory conditions, their size, availability, and developmental similarity to other cephalopods all indicate this species could be an important research animal and also might have potential use as a food source. Until recently such a cephalopod has not been available but now there seems to be a small choice between several decapod species (Boletzky *et al.*, 1971; Choe, 1966; Richard, 1966; Schröder, 1966; and La Roe, personal communication). *Euprymna scolopes,* living at a uniform temperature near room temperature (24° C) and fairly tolerant of variation of salinity and temperature fluctuations seems to be ideally suited as a laboratory cephalopod. Although relatively unstudied, several areas of investigation could be profitably pursued with this animal. The eyes and brain are relatively large and easily accessible, the egg and embryo are large and easily manipulated and probably could be artificially cultured by the technique of Marthy

(1970) or Arnold (1965b). The potential for ethological studies seems great because the newly hatched squid has a repertoire of behavior patterns some of which, such as burrowing, are intrinsic. We intend to pursue the rearing and culture of this animal in hopes of making it more broadly available.

SUMMARY

Observations were made on the normal development of *Euprymna scolopes* from laying until spontaneous hatching, a period of 20 days at 24° C. Development was very similar to that of *Loligo pealei* with the exception of appearance of gaps between the blastomeres during early cleavage, modifications of the internal and external yolk sac, the closely applied chorion, the early pigmentation of the eye, and the precocious development of the Hoyle organ. Hatching involved several steps, including stretching and pushing with the arms to apply the Hoyle organ to the inside of the chorion. The newly hatched animals ate mysids and attempted to bury themselves in the sand within a few days after hatching. Postembryonic growth was rapid and the animals survived well under laboratory conditions.

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ADDENDUM

Since this paper was submitted for publication, two of the original embryos which hatched in the laboratory survived to sexual maturity, a period of 183 and 202 days, and then died without reproducing. Both were females with fully matured eggs. In addition, LaRoe has published his results on successful laboratory rearing of *Sepioteuthis sepioidea* and laboratory maintenance of *Doriteuthis plei* (Mar. Biol. 9: 9 - 25; 1971).

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